which bubbles of air appeared, proving a patent eustachian tube.

In 2 cases the possible styloid process, which resembled a terminal phalanx of the little finger, had to be removed before the middle ear could be found; there was no bone between the new meatus and the temporomandibular joint and only a partial bony floor to the middle ear. Because of this it was impossible to prevent stenosis of the new meatus but both these children luckily have an apparent marked hearing improvement following the operation.

In 4 cases a fused ossicular mass was found in contact with a mobile stapes in an oval window. In several cases an ossicular mass, consisting of a fused malleus and incus, was found to have no connexion with the stapes and in one of them the floating mobile incus was connected to the deformed footplate of the stapes with a steel piston. In another a perforation was made in a fixed footplate and a piston was used to complete the chain.

The Radcliffe figures show that 75% of the children with bilateral atresia were suitable for surgery and in 75% of their ears it was possible to construct a sound conducting mechanism.

It is difficult to give the hearing results in this series as the children are too young for audiograms and no improvement in hearing can therefore be measured. However, in many of the children, parents and teachers have noticed improvement in speech and mental alertness since operation.

If full use is made of all the information that is being collected about the effect of thalidomide on fœtal development and if there is a follow-up of all the lines of investigation that have been initiated by the episode, perhaps much may be learned about other teratogenic factors. In this way the tragedies brought about by thalidomide may to some extent be mitigated.

REFERENCES
Wigglesworth R
(1962*a*) *Brit. med. J.* i, 1758; (1962*b*) *Lancet* ii, 349

Principles of Teratogenesis: Mode of Action of Thalidomide

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When a pregnant animal ingests a noxious substance the developing young are exposed to a wide variety of hazards. The substance may reach the embryos and poison them directly in the same way as it does the adult who takes it; the response of the embryo to the stimulus may, however, differ from that of the adult. First of all, embryonic tissues are generally more sensitive to the effects of toxic substances than are those of the adult: this is indicated by the fact that, in the human, the abortion of dead and deformed embryos is more common than the recovery of a perfect living embryo from a dead mother. Secondly, embryonic cells must not only survive but must also differentiate and reproduce themselves according to a strictly regulated and predetermined pattern; if this process is hindered malformations will result. The embryo may be indirectly vulnerable to the influence of noxious substances or poisons which may act on the mother in such a way as to interfere with the normal conditions required for its proper development: contraction of the uterus may dislodge it from its site of implantation; interruption of blood flow may deprive it of oxygen; poisoning of the maternal liver and other organs may present it with toxic metabolites or reduce the available supply of substances required to build up the embryonic tissues. Thirdly, intervening between the mother and the fœtus is the placenta, derived from both but acting in important ways as an independent structure. Before the implantation of the blastocyst a teratogen can kill the embryo but is powerless to deform it.

The final effects of a drug on the embryo can be described in simple terms. The embryo is either unaffected and born in a healthy normal state or, if affected, may be born alive and deformed or dead and deformed. Indeed, it may be aborted immediately on exposure to the effects of the drug. These simple effects are, however, the results of complex processes. In Table 1 are set out the possible sites of action of factors which may produce malformations in the mammalian fœtus.

Table 1
Possible sites of primary action of genetic and environmental factors

(1) *On the maternal tissues*
Sites of action:
Circulating blood, liver, kidney, brain, heart, uterus, ductless glands
Modes of action:
(*a*) Reduction in oxygen-carrying capacity of blood
(*b*) Alteration in the level of blood glucose
(*c*) Reduced availability of essential vitamins, hormones, amino acids and trace elements

(2) *On the placenta*
Modes of action:
(*a*) Interference with passage of oxygen, glucose or other vital substances across the placenta
(*b*) Interference with blood supply of placenta

(3) *On the embryo*
Modes of action:
(*a*) Direct effect on the cells in the structure which is to be deformed
(*b*) Action on embryonic liver
(*c*) Action on fœtal heart and circulation
(*d*) Action on embryonic endocrine system

It is important to recognize that, at the various sites indicated, the action of a teratogen as the prime deforming factor may be supplemented by other factors, genetic and environmental, operating at this and other sites, providing an explanation for differences in the response to teratogens between members of the same species or even the same litter. The existence of these various sites for possible teratogenic action must be borne in mind when assessing a new theory of the action of one or all teratogens: for example, actinomycin may exert its teratogenic properties through its power of inhibiting DNA-dependent RNA synthesis, probably by combining with the RNA template (Shatkin *et al.* 1962), but this still leaves open the question of where this activity takes place; we still need to know whether the crucial effect is on the tissues of the mother, the fœtus or the placenta. On the other hand, the postulate that vitamin A owes its teratogenic activity to an ability to raise the protease activity of chondrocytes by increasing the permeability of their lysosomes must directly relate the effect of the teratogen to the tissue which is later found to be malformed.

Because thalidomide is, we hope, the only teratogenic substance to exert a major effect on human development, there has been a tendency to regard it as in a category of its own, quite separate from the other agents which are known to produce malformations in the young of experimental animals. From the biological standpoint cortisone is a much more effective teratogen than thalidomide, since it can produce 100% incidence of malformation in the mouse, a level which has not been reached even with extremely high dosages of thalidomide.

To estimate the significance of thalidomide as a teratogen it is necessary to know something of the history of mammalian teratology prior to the thalidomide disaster. Most of the important discoveries have come by accident when malformations were found in the young of animals subjected to some noxious agent for experimental purposes. The factors determining the further investigation of any particular agent have been the ease of producing deformities with that agent and the type of malformation produced. If, for example, a deformity is produced which is instantly recognizable, such as exencephaly, the agent is bound to be the subject of much fuller investigation than if it had produced a malformation such as coloboma of the eye, diaphragmatic hernia, valvular defect of the heart or some other deformity which would require for its identification the laborious preparation of box after box of serial sections on slides. The conditions under which teratogenic experiments have been carried out have also excluded defects which manifest themselves as biochemical dysfunctioning and considerations of space have generally prevented young, born to dams subjected to possible teratogens, from being allowed to survive to adult life, so that defects of mental behaviour or reproductive ability, minor malformations of the lung, kidneys and heart have not been the subject of investigation.

Much of the information which has revealed the basic principles of teratogenesis as they are understood today has come from the use of a few agents such as X-radiation, cortisone, hypovitaminosis-A and hypervitaminosis-A, which are easy to administer under laboratory conditions. The use of these agents has made the experimentation highly selective in another way, namely, in the species of animals used. A suitable teratogenic dose of X-radiation in the critical period of pregnancy for many, if not all, mammals would be 200 to 300 r. The problems of delivering that quantity of whole-body radiation rule out the use of an animal such as the elephant, and virtually also the cow and pig. It is not surprising to find that the studies on X-radiation as a teratogen were carried out in rodents and that even amongst rodents much more work has been done in mice than in rats. Fraser and his colleagues have performed their investigations of the teratogenic activity of cortisone solely in the mouse. At a recent conference attended by workers from a large number of pharmaceutical firms who were actively engaged in teratogenic testing, it was found that nobody was carrying out experiments using mammals other than rats, mice and rabbits. Such principles of teratology as we have ought therefore to be styled the principles of *rodent* teratology and we have always to bear in mind the possibility that the extrapolation of these principles to the human situation may be no more justifiable than the extrapolation of the results of experiments on the amphibian embryo or hen's egg to explain the phenomena of mammalian teratology, an exercise which succeeding generations of embryologists have found decreasingly profitable.

Teratogenic Agents

In mammalian teratology the time scale can really be divided into two eras, 'before thalidomide' and 'after thalidomide'. Before thalidomide the amount spent in this country annually on research into mammalian teratology was not quite sufficient to maintain two students at a university. Today I estimate the pharmaceutical industry to be spending on teratogenic experiments, directly and indirectly, at least enough money each year to set up a new university in this country. Not surprisingly, the literature on teratology is now becoming swamped by a mass of information on

the teratogenic effects of pharmaceutically interesting compounds. A list of the drugs known to produce embryopathy, that is death or malformation or both, in experimental animals (Table 2) shows that these substances can be divided into a number of groups: antimetabolic agents, hormones, vitamins and their antagonists, antibiotics, alkaloids, leaving a number of other substances including thalidomide which may or may not be classifiable in future under one of the previous subheadings when we know more about their mode of action.

Table 2

Drugs capable of producing embryopathy in laboratory mammals

(1) *Antimetabolic and antineoplastic agents*
(*a*) Alkylating agents: Nitrogen mustards, busulphan, chlorambucil, triazine derivatives, tretamine, thiotepa, mannomustine, E-39 soluble, TEPA, 2-amino 1,2,4 triazole
(*b*) Actinomycin
(*c*) Colchicine
(*d*) Vitamin antagonists: Antagonists to nicotinamide, pyridoxine, folic acid, pantothenic acid, vitamin B_{12}
(*e*) Antagonists to nucleic metabolism: 6-chloropurine, 6-mercaptopurine, 2,6, diaminopurine, 8-azaguanine, thioguanine, 5-fluoroorotic acid, 5-bromouracil, 5-fluorouracil, desoxyuridine, desoxycytidine, diazooxonorleucine, azaserine, pyrimidine analogues
(*f*) Amino-acid antagonists: Methionine sulphoximine, ethionine, methionine sulphoxide
(*g*) Alazopeptine

(2) *Hormones*
(*a*) Anterior pituitary extract, ACTH, anterior pituitary growth hormone, thyrotrophin, prolactin, posterior pituitary extract
(*b*) Thyroxine
(*c*) Insulin, glucagon
(*d*) Cortisone, prednisolone, hydrocortisone and derivatives, desoxycorticosterone, aldosterone
(*e*) Testosterone, methylandrostenediol, androsterone, dehydroandrosterone, &c.
(*f*) Œstradiol, progesterone, &c.
(*g*) Adrenaline
(*h*) Chorionic gonadotrophin

(3) *Vitamins*
(*a*) Vitamin A
(*b*) Vitamin B_{12}
(*c*) Vitamin D

(4) *Antibiotics and bacteriostatic sulphonamides*
Penicillin, streptomycin, tetracycline, oxytetracycline, polymyxin B, various sulphonamides

(5) *Alkaloids*
Caffeine, ergot, ergotamine, podophyllin, quinine, reserpine, pilocarpine

(6) *Hypoglycæmic sulphonamides and biguanides*
Carbutamide, chlorpropamide, tolbutamide, glybuthiazole, N.N. Dimethylguanylguanidine

(7) *Thalidomide*

(8) *Antihistamines*

(9) *Chlorpromazine*, prochlorperazine, promazine, acepromazine, thiodinazine, thioridiazine, levopromazine

(10) *Salicylates*

(11) *Thiourea*, methylthiouracil, propylthiouracil, iodothiouracil

(12) *Various:* Acetazolamide, lysergic acid amides, usnic acid, Aprobit, bretylium (tosylate), chlordiazepoxide, chlorothiazide, cyclizine, cyproheptadine, cysteamine chlorhydrate, dicoumarol, dimercaprol, furazolidone, glutethimide, guanethidine, imipramine, L.1935, mepacrine, meprobamate, mepyramine, methaqualone, methazolamide, a Methyl DOPA, nialamide, 5-nitro-2-furaldehyde-2 (2-hydroxyethyl) semi-carbazone, phenobarbitone, phenylbutazone, spironolactone, thymidine, trifluoperazine, triparanol, urethane, ethylurethane

The contemplation of this list makes one wonder whether there is any effective medicine which, if it were tested with sufficient thoroughness, would not appear on the list. Why is it that of all these substances thalidomide is the only one to have produced a disastrous epidemic of malformation in the human community? The answer is not simple. There is regrettably little information upon the risk to the human fœtus of the mother taking thalidomide at the critical period of pregnancy, since at the time of the thalidomide disaster, world-wide lack of interest in and support for teratological research meant that vital information simply was not obtained. It would seem from Japanese and other figures that the risk to the human fœtus was not less than 25%. It was certainly not 100%, for a number of well-authenticated cases are known of women who took thalidomide at the critical period and produced perfectly normal healthy children. At least two drugs, actinomycin and aminopterin, and probably others, might well have shown much more drastic teratogenic activity had their therapeutic properties brought them to use at the critical time in a large population of pregnant women. Since the incidence of easily recognizable malformations is not less than 2% there are bound to be many occasions when a woman who takes a drug during the critical period of pregnancy gives birth to a malformed child. Only in the case of thalidomide and aminopterin has a causal relationship been shown to exist between the two events.

Factors Capable of Influencing the Activity of Teratogens

The effect of a teratogen is modifiable by other factors. The actions of teratogens may summate and the effects may be modified experimentally, increased or decreased, by the simultaneous administration of other substances (Woollam & Millen 1960). There are a number of agents, ranging from cabbage taken in the food to metabolic agents, physical agents such as stress, starvation, noise, hypothermia, hyperthermia and hypoxia, all of which one imagines could act together with a teratogen to increase its effects. Of such adjuvant factors undoubtedly the most important is that mixed bag of characteristics, identified or unidentified, which we group under the heading 'genetic factors'. It appears that some mothers took a number of thalidomide tablets with impunity, others produced a malformed offspring after a single tablet. We know very little about the way in which thalidomide exerts its teratogenic effects. They may be mediated through its main metabolic products, which are derivatives of glutamic acid and glutamine and may act as competitive antago-

nists to these substances. On the other hand, thalidomide shows an antimitotic effect on the growth of human leucocytes in tissue culture; this may or may not reflect a role as a glutamic acid and glutamine antagonist. Nevertheless, accepting that its activity depends on such mechanisms, there are a number of possible ways in which these effects may be modified. The drug is taken by mouth and is not very soluble so that a considerable individual variation in the amount absorbed is likely. Furthermore, some individuals metabolize drugs slowly, leading to their excessive accumulation with resulting toxic effects; whilst others metabolize them extremely rapidly. Chronic administration of drugs can lead to increased metabolism by liver enzymes and to increased metabolism of a subsequent dose of the same or another drug. The possibility thus exists that factors such as constipation, a highly motile digestive tract, abnormal levels of gastric acidity, malfunctioning of the liver or the previous administration of the same or other drugs, may all contribute in determining whether a woman who took thalidomide in the critical period of pregnancy, between the 35th and 44th days, produced a malformed or a normal baby.

The Mechanisms of Teratogenic Action

A problem which troubles teratologists at the present time is revealed by the hypothesis which states that teratogenic agents each produce one of a number of possible patterns of malformation and that the choice of pattern depends on the particular metabolic phase with which the teratogen interferes. Is this hypothesis true or false? Runner (1959) has produced evidence in the mammal which shows that very similar deformities result from the use of folic acid antagonists, iodoacetate, fasting or insulin. He suggests that all these agents interfere in the citric acid cycle, probably at different levels. It has been postulated that other teratogens interfere with other aspects of the Krebs cycle. Over 35 teratogens have been shown experimentally to produce limb deformities (Millen 1962). It is commonly agreed that, in man, thalidomide chiefly attacks the skeletal tissues and a similar propensity has recently been shown for the monkey, *Macaca irus philippiensis*, in which anotia has recently been produced by the administration of thalidomide to the pregnant dam (Delahunt & Lassen 1964).

One of the difficulties we have to face when endeavouring to elicit the mechanism of action of a teratogen is the number of levels at which the activity can be regarded as taking place. We not only have to consider the possibility that teratogens such as thalidomide may act on the tissues of the mother, the embryo or the placenta, we have also to analyse more fully what we mean by action on the tissues. Let us assume that thalidomide acts directly on the embryo and has an affinity for skeletal tissues. We have then to decide whether it is most important to investigate its effect on the tissues at the molecular level, the level of the organelle or the level of the cell. At the first level we shall be investigating possibilities such as the inhibition of RNA synthesis; at the level of the organelle we shall be studying, for example, whether the drug affects the lysosomes, Golgi apparatus or mitochondria; at the cellular level we shall be interested in its effects on the division of the cell. There are five possible end-results of the effect of a teratogen at the cellular level: it can stimulate cell division; it can leave a cell unharmed; it can slightly affect the cell, slowing down the rate at which it divides; it can stop it dividing; it can kill it. In practice, then, the effect of a teratogen is to prevent the cell from being in the right place doing the right thing at the right time, so that, for example, two components which should meet exactly to separate two chambers fail to do so, leaving a patent ventricular septum, a diaphragmatic hernia or a cleft palate. It would seem that, in general, the effect of a teratogen is to kill cells without killing embryos, thus depriving the developing organ not only of a single cell but of all its descendants as well and, even more subtly, of the microstructures that it would normally evoke.

The problem is even more complicated, for cell death *per se* is not an abnormal occurrence in the embryo; on the contrary it is one of the vital forces modelling the form of the tissues. In the central nervous system, for example, many neuroblasts die as a perfectly normal feature of the developmental process; the meaning of this phenomenon is far from clear; we do not know whether the cells which are discarded are certain specific cells which die because they are no longer needed, as nerve cells die in amphibians when the transition from the tadpole stage occurs, or whether they are undifferentiated cells which happen to be expendable. It has been argued that the mammal possesses just as many nerve cells as he can supply with oxygenated blood and no more (Woollam 1962) and that the effect of cell death is, as it were, to scrape away the surplus. Be that as it may, the specific action of a teratogen is to interfere with the division of cells which are *not* expendable.

We know very little about the factors which serve to make a cell vulnerable to the action of a teratogen but what we do know points to the probability that cells are most vulnerable when they are most actively dividing, that is, at the period of greatest mitotic activity of an organ. By a curious misconception those unfamiliar with

embryonic tissues are apt to imagine that at any one time the embryo is teeming with cells in a state of active division. If one looks at a series of slides of a developing embryo, only rarely does one see a cell in metaphase. The explanation lies in the fact that, complicated as the human fœtus at birth appears to be, it nevertheless contains a number of cells, approximately fifteen million million, which could be produced by only seventeen divisions from the zygote, assuming division of every member of each generation of daughter cells. This does not, in fact, take place and division proceeds at an unequal rate as between tissues. Metaphase, however, only occupies about forty-five minutes, so that one can rarely expect to encounter cells at this phase in the embryonic tissues. Since the relative vulnerability of two organs to a teratogen may reverse itself within a period of twenty-four hours, a teratogen could thus produce its effect by interfering with a small number of cells over a short period of time.

Future Research

To elucidate the mechanism of action of thalidomide and to make it possible to know whether a new drug is likely to produce malformations in the human there are a number of very simple pieces of information we require, such as the normal incidence of malformations in the human population and in colonies of laboratory animals. This information would enable us to select for study an animal whose malformation rate closely corresponded to that of the human. We need prospective studies of as many human pregnancies as possible. If these studies had been in full swing at the time of the thalidomide disaster, not only might the number of thalidomide-induced malformations have been greatly reduced but also a great deal might have been learnt which would have been extremely valuable in determining why human malformations occur under normal circumstances. As I have indicated, we need fundamental experimental research by workers in a wide variety of disciplines studying the effects of teratogens on *inter alia* DNA-based RNA synthesis, distribution and activity of intracellular organelles, mitosis and meiosis, cell populations in mammalian embryos, maternal, fœtal and placental chemistry, abortion, stillbirth and malformation rates in experimental animals, the mechanisms of normal and abnormal organogenesis.

REFERENCES
Delahunt C S & Lassen L J (1964) *Science* **146**, 1300
Millen J W (1962) *Lancet* ii, 599
Runner M N (1959) *Pediatrics, Springfield* **23**, 245
Shatkin A J *et al.* (1962) *Biochem. biophys. Acta* **55**, 227
Woollam D H M (1962) *Clin. Physiol.* **4**, 1
Woollam D H M & Millen J W (1960) In: Ciba Foundation Symposium on Congenital Malformations. Ed G E W Wolstenholme & C M O'Connor. London; p 158

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Papers

An Ultra-structural Analysis of Vestibular End-organs with Special Reference to a Unit Analysis of their Function [*Abstract*]

by Professor O E Lowenstein DSC FRS
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Electrophysiological studies of the cristæ and maculæ of the fish labyrinth have yielded exhaustive qualitative and quantitative information on the mode of response of the hair cells which make up these sensory epithelia. Little is known, however, about details of the process of transduction of the mechanical deformation of the sensory hair processes into the pulse-coded signal ascending the myelinated fibres of the sensory nerve. Our ignorance in this field is partly due to the difficulties encountered in attempts to penetrate the hair cells themselves with micro-electrodes. Ultrastructural features may therefore at present furnish the only useful clues. The results of a complete survey of the topographic arrangement and morphology of the hair cells of the elasmobranch *Raja clavata* were reported and discussed with reference to their function as mechano-electric transducers. It was claimed that these considerations were immediately applicable to human vestibular physiology.

REFERENCES
Lowenstein O (1956) *Brit. med. Bull.* **12**, 110, 114
Lowenstein O, Osborne M P & Wersäll J (1964) *Proc. roy. Soc.* Ser. B **160**, 1

DISCUSSION

Professor F C Ormerod (*London*) said that he would like to answer, as far as he could, Professor Lowenstein's question about the relation of the cochlear hair processes to the tectorial membrane. It seemed to be generally accepted that the hair processes of the sensory cells of the crista and macula in the vestibule penetrated the cupula and the otolith membrane. In the cochlea the hair processes of the sensory cells of the organ of Corti had, for many years, been described by Retzius, by Held, by Shambaugh and others and more recently by Borghesan, all using the light microscope, as projecting into channels in the mass of the tectorial membrane. Some three